Esp

Week 15

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Transformation of pSB1C3-T7-EB into BL21(DE3), liquid culture of the transformed cells and plasmid purification

Responsible

Aman Mebrahtu

Protocols used

- Transformation - Colony picking

Modifications and comments to protocols

Liquid cultures in 10 ml LB media with 25 ug/ml chloromphenicol

Results and Conclusions

After transformation, one colony developed. We purified the plasmid from the liquid culture, which resulted in a concentration of 40.4 ng/μl. The plasmid was sent for sequencing, whose barcode number was 90AA54.

Discussion and Troubleshooting

The experiment was successful. We are waiting for the sequencing result.

2 Transformation of pSB1C3-T7-EB into BL21 cells

Responsible

Aman Mebrahtu

Protocols used

- Transformation

Results and Conclusions

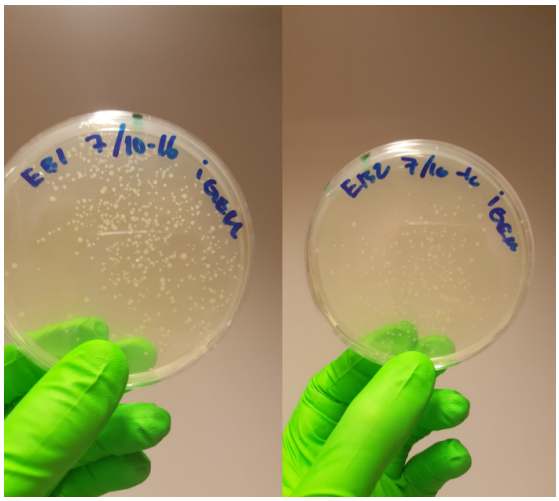

Figure 1: The BL21 cells transformed with pSB1C3-T7-EB in duplicates.

As shown in Figure 1, the transformation was successful.

3 Addition of BamHI restriction site downstream to the EB sequence and amplification of the whole plasmid, using overhang PCR - first attempt

Responsible

Aman Mebrahtu

Protocols used

- PCR
- Gel electrophoresis

Experimental Set Up

The purpose of the overhang PCR was to amplify the whole pSB1C3-T7-EB construct with the addition of a BamHI restriction sequence after the EB coding sequence. This BamHI site serves as a place of attachment for linker tags, which will be added later on. To maintain accuracy of the PCR performed, we chose to work with Phusion High Fidelity DNA Polymerase. The PCR reaction composition as well as the PCR conditions are written in tables below.

Table 1: PCR reaction of Overhang EB

Reaction	Volume [μl]
Nuclease-free water	10.8
5X Phusion HF	4.0
10 mM dNTPs	2.0
10 μlM Forward Primer	1.0
10 μlM Reverse Primer	1.0
Template DNA	1.0
Phusion DNA Polymerase	0.2

Table 2: PCR condition using Phusion DNA polymerase

Step	Cycles	Temperature {°C}	Time
Initial Denaturation	1	98	30 secs
Denaturation	30	98	10 secs
Annealing		60	30 secs
Extension		72	1.5 min
Final Extension	1	72	10 min
Hold	indefinitely	4	-

Results and Conclusions

The PCR was unsuccessful. No clear bands appeared in the gel.

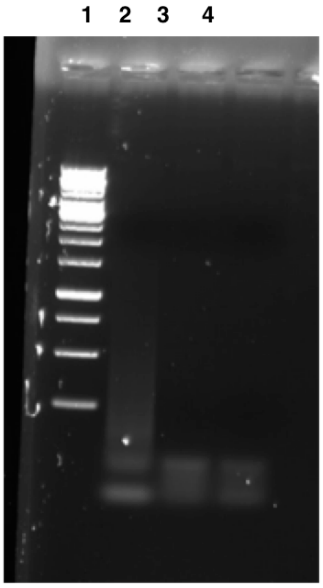

Figure 2: PCR result. (1) DNA ladder (2) EB with EcB Phusion primer (3) EB with EbB Phusion primer (4) negative control

Discussion and Troubleshooting

The failure could be due to several reasons: the PCR program or conditions might not be successful, or the primer might not be compatible. We will try new conditions in the following attempts.